

Annual Reproductive Cycle of Two Japanese Species of Sipunculans: *Siphonosoma cumanense* (Sipunculidae) and *Phascolosoma scolops* (Phascolosomatidae)¹

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ABSTRACT: The annual reproductive cycles of the Japanese sipunculans *Siphonosoma cumanense* (Keferstein) and *Phascolosoma scolops* (Selenka, de Man & Bülow) were studied based on size, density, and frequency distribution of gametes from May 1989 to November 1990 and October 1989 to February 1991, respectively. Aliquots from the total volume of the diluted coelomic fluid from each individual were analyzed. Coelomic oocytes of *S. cumanense* showed a slow growth rate (i.e., 3 $\mu\text{m}/\text{month}$) from December to April. An accelerated rate was observed in May to July. No oocytes were encountered from September to December, suggesting that spawning occurs in July to August. Sperm clusters were encountered only from May to August, with peaks in June and July. In contrast, oocytes and sperm clusters of *P. scolops* were present throughout the year. From January to March small oocytes grew at an average rate of 10 $\mu\text{m}/\text{month}$. Oocytes showed rapid growth until July. Smaller oocytes were noted thereafter, indicating a summer spawning. Fluctuation of sperm cluster density showed a pattern similar to that of oocytes. Both species showed two major peaks in gamete density, one during oocyte proliferation and one before spawning. Stages of gametogenesis in both species are described.

IN SIPUNCULANS, gonads develop up to the first meiotic prophase stage in the gonad, after which they are released into the coelom where they undergo growth. Before spawning, the mature gametes are accumulated into the nephridia where they are stored for a short time before expulsion into the surrounding seawater (Rice 1975). In some species (*Golfingia pugettensis* Fisher [Rice 1975], *Golfingia vulgaris* (De Blainville) [Gonse 1956a, b], *Phascolosoma agassizii* Keferstein [Rice 1966, 1975, Towle and Giese 1967]), coelomic gametes are present throughout the year, whereas in other cases (e.g., *Phascolosoma arcuatum*), they are present only for a few months before spawning (Green 1975).

In studying the annual reproductive cycle of sipunculans, the usual method is to extract a sample of coelomic fluid by means of a needle and a syringe and count or measure the gametes present. Data obtained in this manner may not give an accurate picture of the reproductive cycle of the animal because the distribution of gametes may be uneven within the coelom or the bore of the needle may be limiting. Extraction of the total quantity of coelomic fluid and taking samples from it may give a better estimate and could provide absolute rather than relative counts. Although it is a tedious method, it could provide better data. This method was used in our study.

In this paper we present the annual reproductive cycle of two sipunculans, *Siphonosoma cumanense* (Keferstein) and *Phascolosoma scolops* (Selenka, de Man & Bülow), collected from Nishiwaki Beach, Okayama, and Kuroshima Island, Seto Inland Sea of Japan, respectively. Parameters studied were gamete density, oocyte size frequency distribution, and oocyte growth rate.

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MATERIALS AND METHODS

Collection of Animals

Fifteen individuals of *S. cumanense* were collected monthly and sometimes bimonthly from the muddy substratum at the intertidal zone of Nishiwaki Beach, Ushimado. Okayama, Japan. The same number of *P. scolops* was collected from the intertidal zone of Kuroshima Island, Seto Inland Sea of Japan. In this locality the worms were commonly found inside crevices and small holes in the well-sutured sandstone. For both species, the number of females ($n = 7-15$) and males ($n = 7-15$) varied.

The specimens were transported to the laboratory in water-filled buckets. When the specimens were not processed immediately, individuals of *S. cumanense* were kept in a 12 by 15 cm container provided with mud obtained from the collection site; those of *P. scolops* were furnished with rocks or dead shells in a similar container.

Examination of Gametes

Trunk length (from the nephridiopore to tail tip) and body weight of each individual were measured to the nearest 1 mm and 0.1 g, respectively, after the body was blotted dry with tissue paper. Trunk length was used to determine maturity, and body weight was used in determining gamete density (i.e., number of gametes per milliliter of coelomic fluid).

The body was cut crosswise a few millimeters below the nephridiopores, and the coelo-

mic fluid was completely drained into a pre-weighed container and subsequently weighed to the nearest milligram. The fluid was diluted with a volume of seawater (for *P. scolops*, 1 to 8 ml; for *S. cumanense*, 5 to 15 ml). A 0.01-ml subsample of this solution was taken after the sample was thoroughly mixed and its content of gametes examined. For females, size of oocytes (diameter for *S. cumanense* and longest axis [i.e., length] for *P. scolops*) and oocyte density were determined in addition to oocyte stages. For males, although the different stages were identified, the number of male gametes per milliliter of coelomic fluid was obtained by counting all the coelomic gametes regardless of stage because of the difficulty of counting the density per stage. Three subsamples per individual were analyzed.

The size of the gametes was measured using a phase-contrast microscope with an eyepiece micrometer ($200\times$).

The growth curve of the oocytes was constructed according to Gibbs (1975). The average of 10 of the largest oocytes in each female was used.

RESULTS

Anatomy of the Reproductive System

Both species are dioecious. However, no evidence of sexual dimorphism was observed, and sexes can only be determined by examining their coelomic gametes.

The gonad of both species is located along the base of the two ventral retractor muscles. It extends from the lateral edge of one of the

TABLE 1
CHARACTERISTICS OF THE STAGES OF COELOMIC OOCYTES OF *S. cumanense*

STAGE	DIAM. (μm)	AVERAGE THICKNESS OF MEMBRANE (CHORION) (μm)	DIAM. OF NUCLEUS (μm)	DISTRIBUTION OF YOLK GRANULES
1	10-40	0.5	5-12	Fewer in the outer cytoplasm than those around the nucleus
2	41-99	0.8	15-20	More in the outer cytoplasm than those around the nucleus
3	100-119	1.0	25-30	Evenly distributed in the cytoplasm
4	120-127	2.0	35-40	Evenly distributed in the cytoplasm

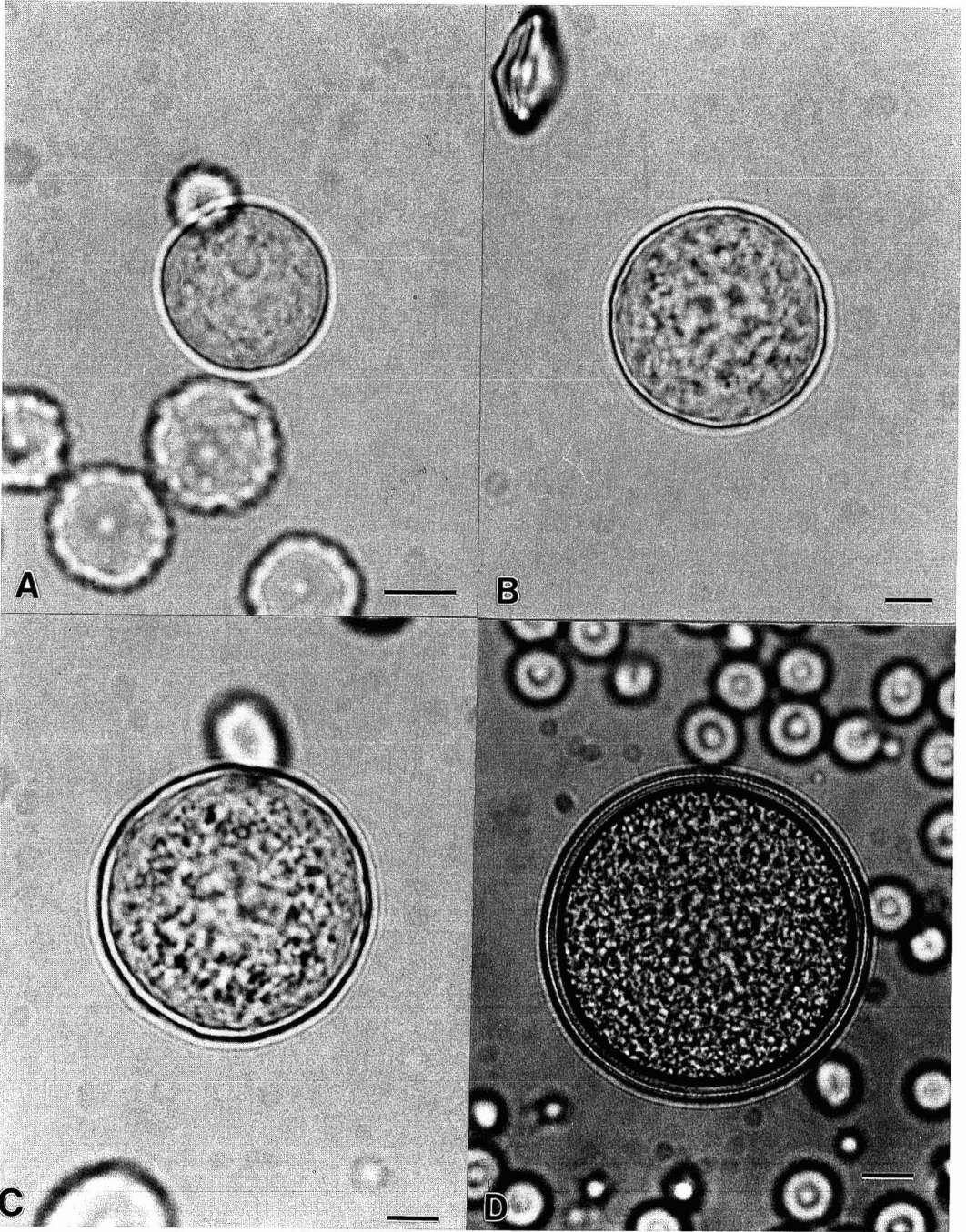


FIGURE 1. Representative stages of coelomic oocytes of *S. cumanense*: *A*, stage 1; *B*, stage 2; *C*, stage 3; *D*, stage 4. Scale = 10 μm (*A*, *B*); 20 μm (*C*, *D*).

muscles, under the ventral nerve cord, to the lateral edge of the other muscle. The ovary and testis are similar in form and color.

The smallest individual with coelomic gametes encountered during the sampling period measured 5 cm and 1 cm in trunk length for *S. cumanense* and *P. scolops*, respectively. The former weighed 2 g, and the latter weighed 56 mg.

Cytological Stages of Oogenesis

S. cumanense: Oocytes of this species are released in the coelom as solitary cells. The characteristics of the different stages of oocytes are summarized in Table 1 and shown in Figure 1A–D. In the last oocyte stage there is formation of a jelly coat.

P. scolops: Coelomic oocytes were never in clusters. The different characteristics of the various stages of oocytes of this species are given in Table 2 and shown in Figure 2A–G.

Cytological Stages of Spermatogenesis

Stages in spermatogenesis have been classified based on size and shape of the sperm clusters and their component cells:

S. cumanense: (1) Stage 1. Spermatocytes break off from the testis as spherical masses. These are 25 to 40 μm across, with each cell

measuring 5 μm in diam. (Figure 3A). (2) Stage 2. The cells within each sphere increase in number and develop into spermatocyte clusters. Spermatocyte clusters measure to 30 μm across; each cell is 2 to 3 μm in diam. With a tail 5 μm long (Figure 3B). (3) Stage 3. This stage consists of clusters of spermatids and spermatozoa, which are indistinguishable from each other. Each head about 2 to 3 μm in diam. and the tail is up to 20 μm long (Figure 3C).

P. scolops: (1) Stage 1. Spermatocyte spheres are about 30 to 60 μm across. Individual cells within the cluster are not distinguishable (Figure 4A). (2) Stage 2. These are the spermatids and spermatozoa clusters, which are about 10 to 30 μm long and 15 μm wide. Each cell is 3 μm in diam. with a tail about 40 μm long (Figure 4B).

Oocyte Size Frequency Distribution

S. cumanense: This species has a short reproductive period lasting from May to August (Figure 5). No coelomic oocytes were found from September to November.

From December to April most of the oocytes were small, 10 to 70 μm in diam. During these months the frequency distribution revealed two size groups: one ranging from 10 to 40 μm and the other from 41 to

TABLE 2
CHARACTERISTICS OF THE STAGES OF COELOMIC OOCYTES OF *P. scolops*

STAGE	DIAM. OR LENGTH (μm)	LENGTH: WIDTH RATIO (μm)	AVERAGE SIZE OF FRINGE (μm)	AVERAGE THICKNESS OF VITELLINE MEMBRANE (μm)	SHAPE	COLOR	AVERAGE DIAM. OF NUCLEUS (μm)
1	10–30	1.0 : 1.0	None	0.3	Spherical	Colorless to pale yellow	7
2	31–45	1.14 : 1.0	6	0.5	Some elongated, with one tapering end	Orange	10
3	46–75	1.2 : 1.0	7	0.8	Ovoid, with one end or both tapered	Orange	20
4	76–94	1.25 : 1.0	8	1.0	Ovoid, usually one end sharply tapered	Orange	27
5	95–119	1.22 : 1.0	10	1.3	Both apices flattened	Orange	30
6	120–149	1.21 : 1.0	12	5.0	Central apical depression	Orange	40
7	>149	1.30 : 1.0	5	6.0	Central apical depression	Dark orange	45

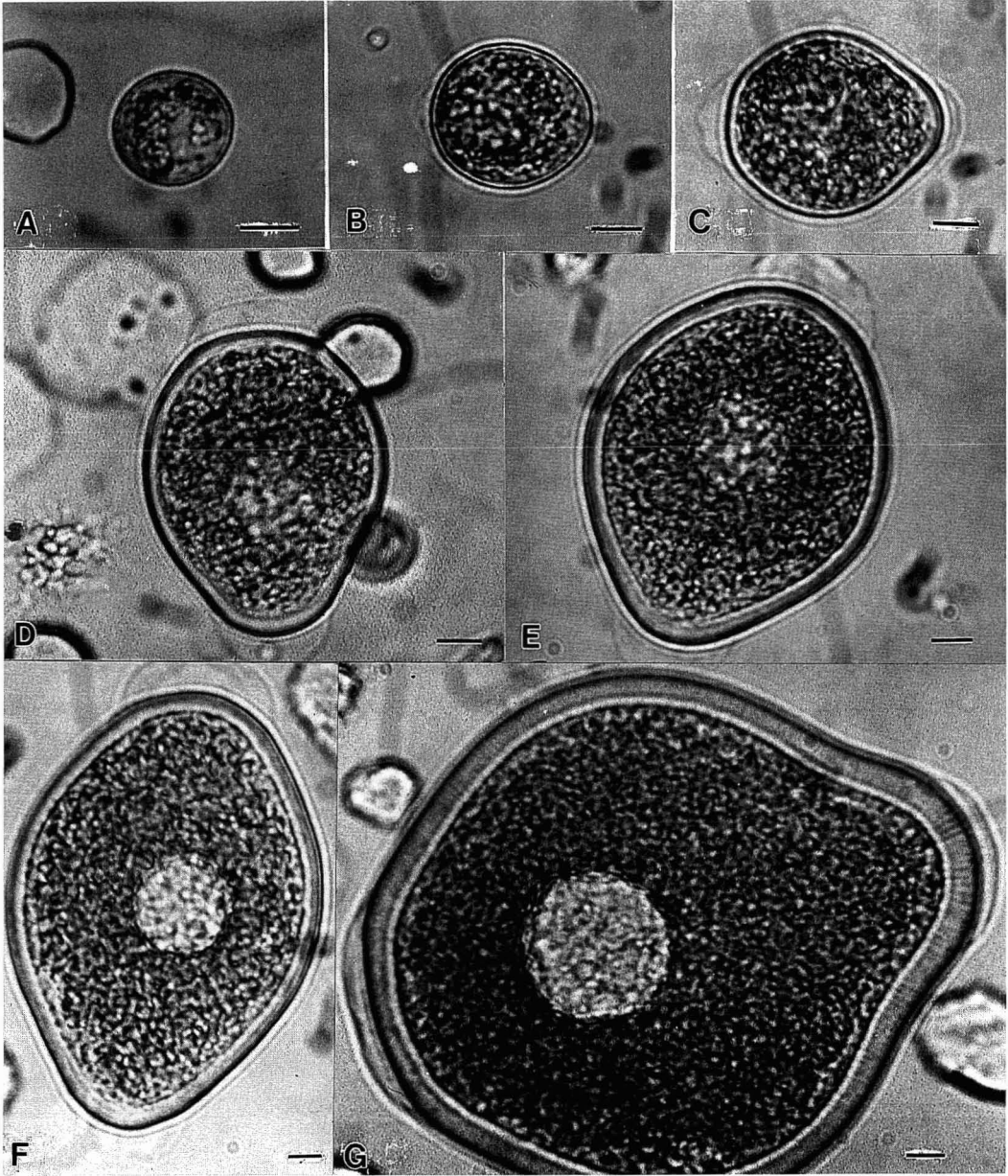
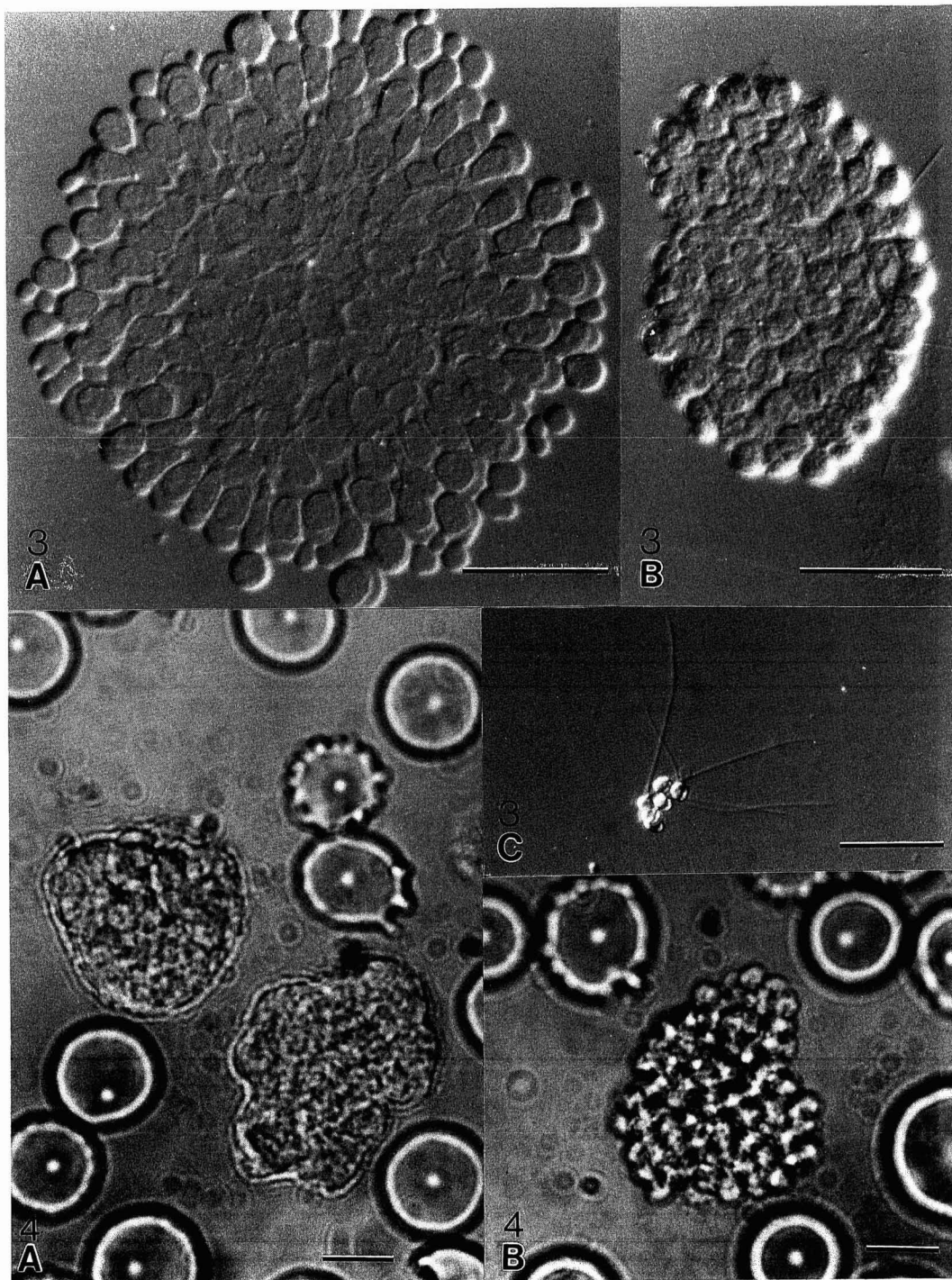


FIGURE 2. Representative stages of coelomic oocytes of *P. scolops*: A, stage 1; B, stage 2; C, stage 3; D, stage 4; E, stage 5; F, stage 6; G, stage 7. Scale = 10 μ m.

70 μ m. Large oocytes appeared in May, increasing the overall size range in coelomic oocytes to 10 to 110 μ m. Fifty percent were larger oocytes ranging from 50 to 110 μ m in diam.

In June 1990, with the appearance of a larger group of cells, the frequency distribution of cells became bimodal, the first at 30 μ m and the second at 90 μ m. Over 50% of the second mode comprised larger oocytes



FIGURES 3–4. 3. Stages of spermatogenesis in *S. cumanense*: A, stage 1; B, stage 2; C, stage 3. Scale = 10 μm . 4. Stages of spermatogenesis in *P. scolops*: A, stage 1; B, stage 2. Scale = 10 μm .

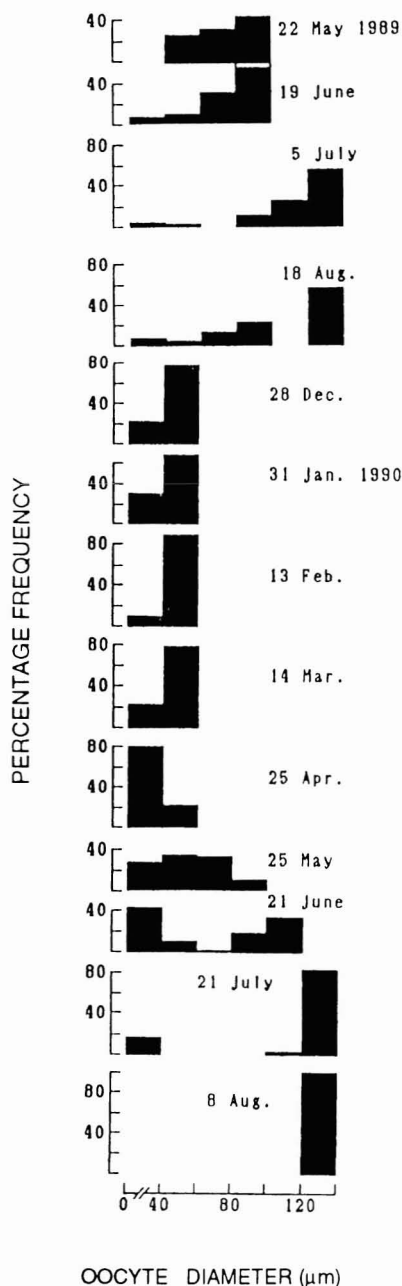


FIGURE 5. Monthly changes in size of coelomic oocytes of *S. cumanense* from Nishiwaki Beach, Okayama, from May 1989 to August 1990. Percentage frequency distribution each month represents measurements of about 50 oocytes from each female ($n = 120$) collected.

ranging from 90 to 120 μm . As the development of the larger oocytes continued, the mode moved to 127 μm in July and was maintained at that level until August.

In 1989, the bimodal distribution appeared from July to August.

P. scolops: Oocytes of various sizes were present throughout the year except in January, when oocytes greater than 130 μm were not present (Figure 6).

The frequency distribution showed small oocytes predominating from September through March. In April and May an even distribution of gametes was observed, ranging up to a diameter of 210 μm . A relative increase of larger oocytes (180 to 210 μm) in June gave rise to a second mode, which was maintained throughout August. A larger-size group (210 to 240 μm) was present only in July.

Oocyte Growth Rate

S. cumanense: The growth curve of *S. cumanense* (Figure 7) is similar to a sigmoid curve. From December to April the growth rate was slow (i.e., 3 $\mu\text{m}/\text{month}$). An accelerated growth of ca. 25 $\mu\text{m}/\text{month}$ was noted from May to July.

The standard deviation of the mean oocyte size was small during the period of slow growth in winter and early spring. Thereafter variation increased gradually at first, then markedly during a rapid growth phase. It eventually decreased as spawning season approached.

P. scolops: In January the largest oocytes were about 60 μm long (Figure 8). Their size gradually increased to about 70 μm in February. In March to July the mean size of the largest oocytes increased to a maximum of 155 μm in length. This represents an average increase of ca. 20 μm monthly during this phase of rapid growth. From August to October the mean size of the largest oocytes gradually decreased to 60 μm once again.

Number of Gametes per Milliliter of Coelomic Fluid

S. cumanense: Females. At the beginning of oocyte proliferation in December, an average

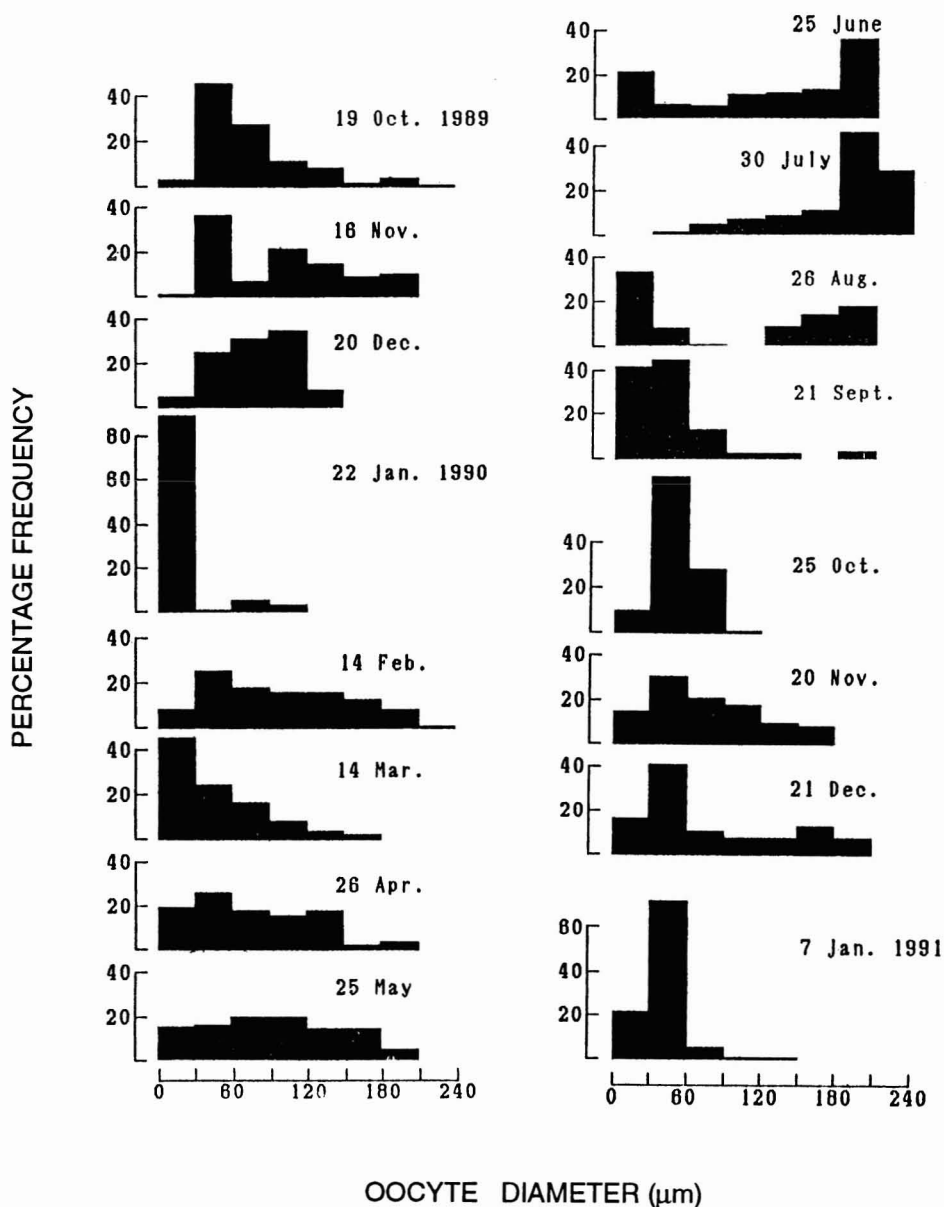


FIGURE 6. Monthly changes in size of coelomic oocytes of *P. scolops* from Kuroshima Island, Seto Inland Sea of Japan, from October 1989 to January 1991. Percentage frequency distribution each month represents measurements of about 50 oocytes from each female ($n = 150$) collected.

density of 300,000 oocytes per milliliter was present in the coelom (Figure 9A). This density decreased in January and February and then rose again until it reached a peak in June.

A sudden drop of about 50% occurred in July, whereas in August, only one female containing about 30,000 oocytes per milliliter was encountered.

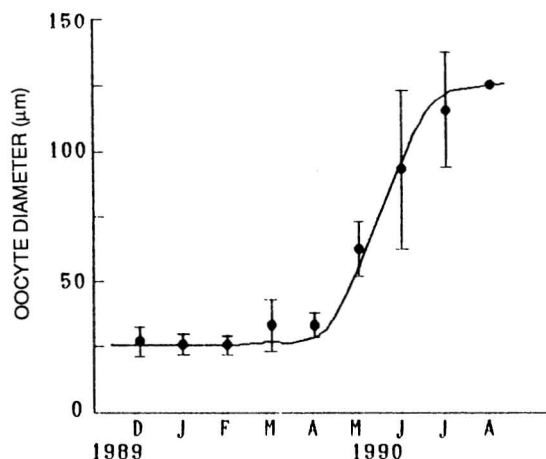


FIGURE 7. The annual cycle in the coelomic phase of oocyte growth in *S. cumanense* collected at Nishiwaki Beach, Okayama. Ten of the largest oocytes in each female ($n = 120$) were measured; mean and standard deviation from December 1989 to August 1990 are shown.

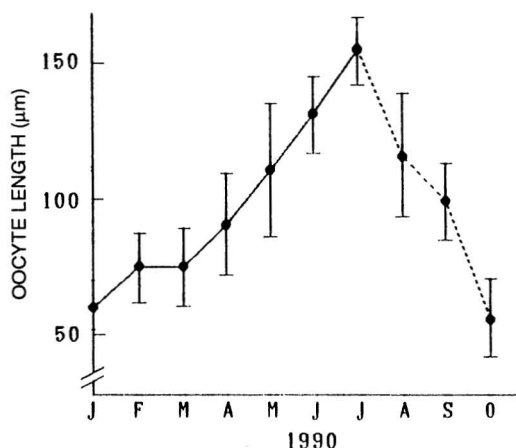


FIGURE 8. The annual cycle in the coelomic phase of oocyte growth in *P. scolops* collected at Kuroshima Island, Seto Inland Sea of Japan. Ten of the largest oocytes in each female ($n = 150$) were measured; mean and standard deviation from January to October 1991 are shown. Dashed lines indicate postgrowth phase.

Males. Sperm clusters were encountered in the coelom from May through August (Figure 9B). In May to June 1989, males contained about 3 million clusters per milliliter. In July the highest peak of about 7.8 million per

milliliter was noted, which eventually decreased thereafter. In 1990, the peak was noted in June.

P. scolops: Females. A small fluctuation in the number of oocytes per milliliter of coelomic fluid was noted among the months except in January and July 1990 (Figure 10A). The highest density (i.e., about 18 million per milliliter) was observed in January, whereas a small peak was noted in July. In 1991, the highest peak occurred in February.

Males. A trend similar to that in females was observed except that the first peak occurred in March (Figure 10B).

DISCUSSION

Reproductive behaviors are often assumed to be intimately related to fitness and under continuous intense selection (Thornhill and Alcock 1983). Because selective pressures vary among habitats, each species develops reproductive strategies appropriate to local environmental conditions. Such species differences in reproductive activities can be seen in this study.

S. cumanense occupies muddy flats, whereas *P. scolops* prefers rocky and sandy portions of the intertidal zone. Although both occupy a similar zone, their microenvironments are different. These differences and similarities in the macro- and microhabitats may explain species distinctness in addition to the innate properties of the species themselves (Odum 1971).

Oocytes have been observed in the coelomic fluid of *P. scolops* throughout the year. In contrast, eggs are absent from September to October or November in *S. cumanense*. The eggs of *S. cumanense* are spherical and remain so throughout the year, whereas the coelomic oocytes of *P. scolops* are first released from the ovary as round cells but change to slightly flattened ellipsoids as coelomic oogenesis proceeds.

Other sipunculans have similarities with these two species. *Golfingia pugettensis* (Rice 1974b) and *Golfingia ikedai* Fisher (Sawada et al. 1968), like *S. cumanense*, have spherical oocytes throughout the year. In all oocytes of

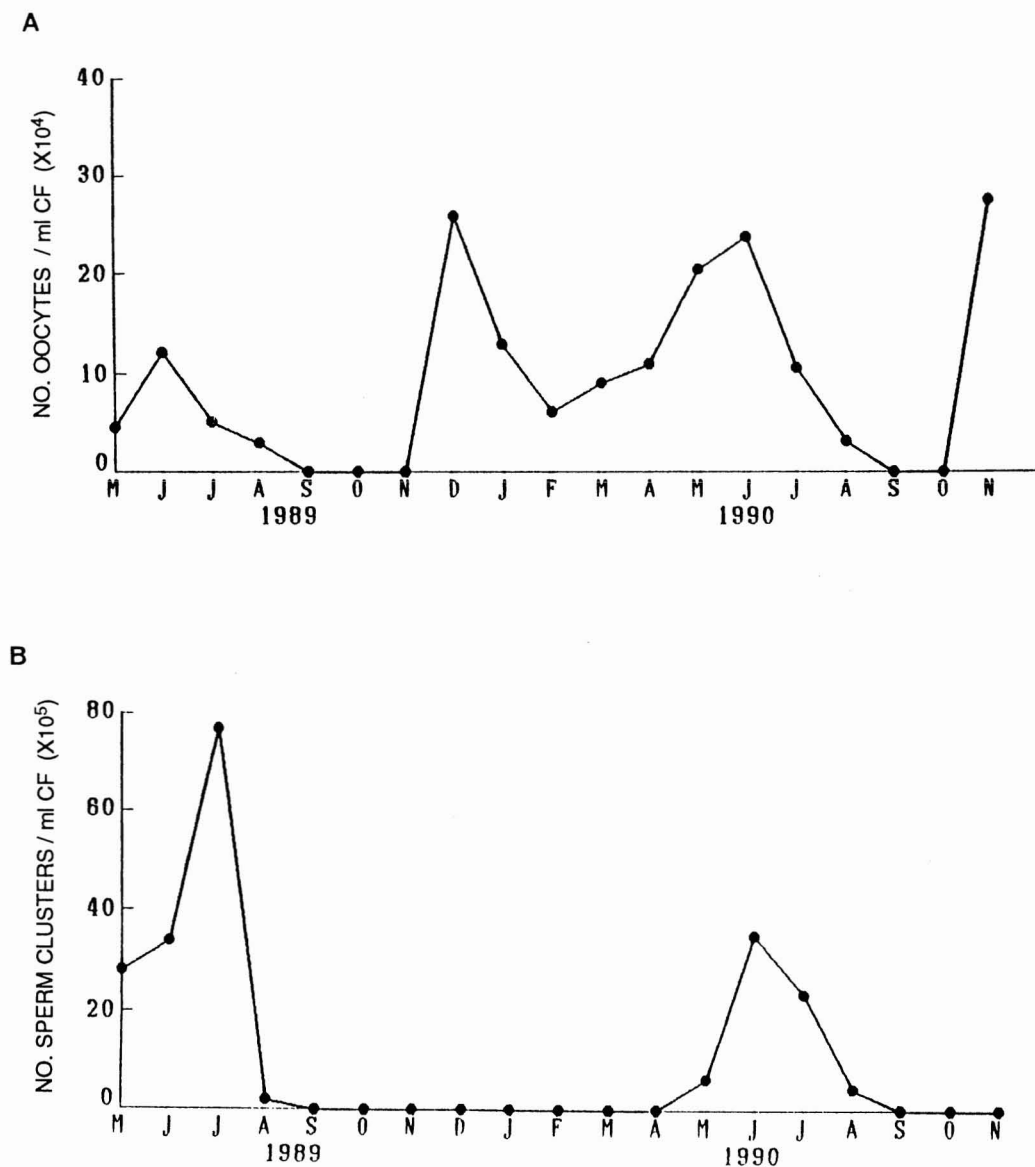


FIGURE 9. The monthly average of number of (A) oocytes and (B) sperm clusters present in the coelom of *S. cumanense* (females: $n = 120$; males: $n = 118$) collected at Nishiwaki Beach, Okayama, from May 1989 to November 1990.

the three species yolk granules are distributed from the perinuclear zone to cytoplasm until the whole cytoplasm is completely covered during oocyte maturation. Similarly, there is an increase in the thickness of the membrane with the formation of a jelly coat at the last oocyte stage.

On the other hand, the coelomic oocytes of *Phascolosoma agassizii* (Rice 1974b) are similar to those of *P. scolops* except for oocyte size range (the former has an oocyte size ranging from 20 to 45 μm long; those of the latter measure from 10 to 149 μm long). Their oocytes are first released from the ovary as

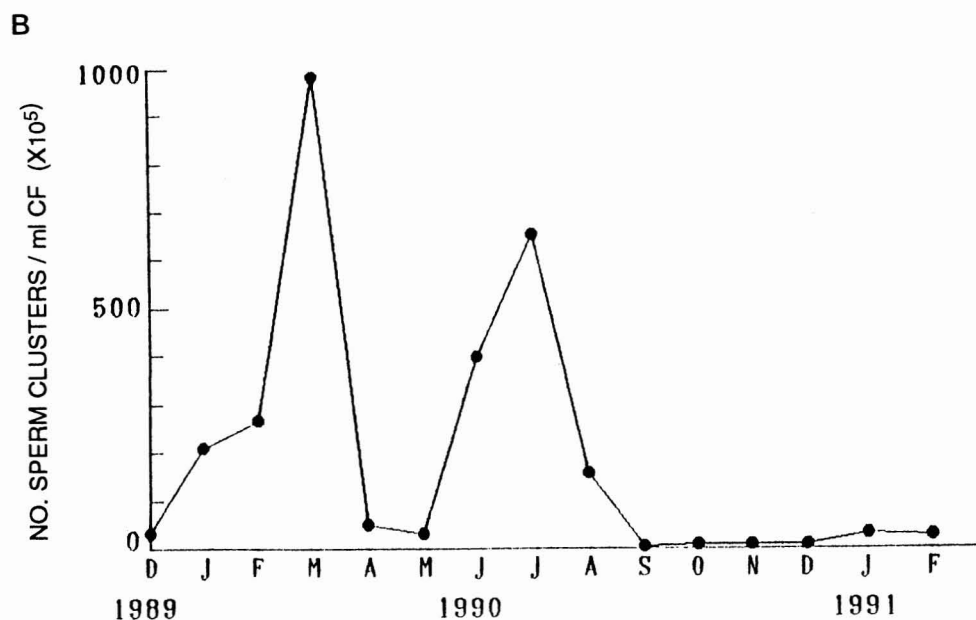
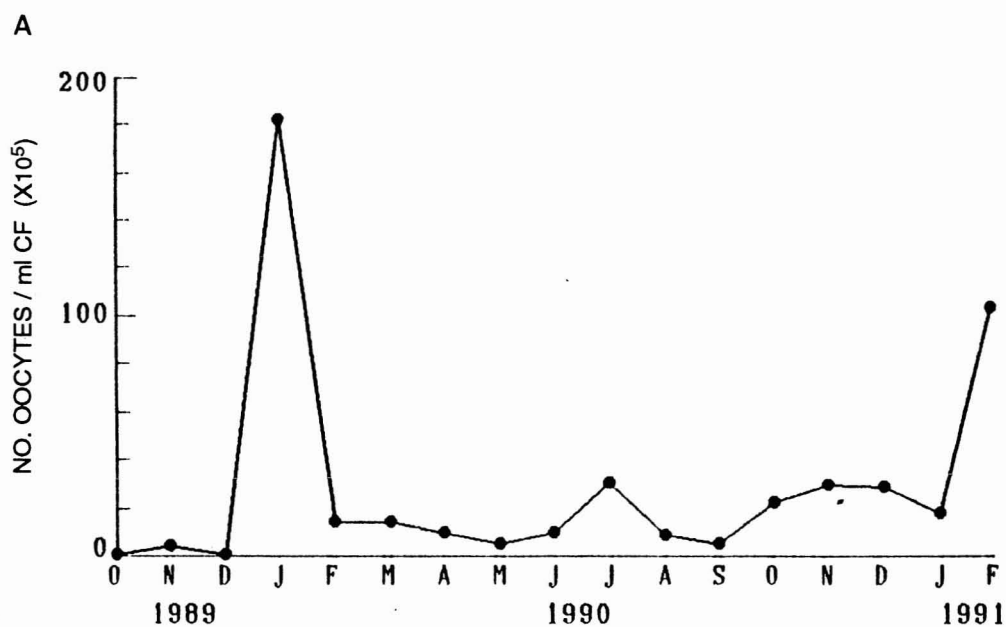


FIGURE 10. The monthly average of number of (A) oocytes and (B) sperm clusters present in the coelom of *P. scolops* (females: $n = 150$; males: $n = 145$) collected at Kuroshima Island, Seto Inland Sea of Japan, from October 1989 to February 1991. (B) has no data from October to November 1989.

round cells and they change to slightly flattened ellipsoids as coelomic oogenesis proceeds. As they become ellipsoidal, a certain polarity is established. Rice (1974b) suggested that the polarity and bilateral symmetry exhibited at this phase is maintained in the embryo. The animal and vegetal poles can be distinguished by the shape of the oocytes, with the latter pole being more tapered. At the same time a fan-shaped structure was noted at the poles. Rice (1974b) suggested that these are tips of microvilli marked by changes of amorphous material, which in older stages coalesce to form a homogenous fringe around the egg, widening at the poles. Both species are also similar in the characteristics of the various stages of spermatogenesis, with free spermatozoa being uncommon in the coelom.

Phascolosoma arcuatum (Green 1975) is similar to *S. cumanense* in terms of the presence of sperm in the coelom for only a short time before breeding. Both species have a similar length of spawning time (2 months) but differ in the season. The former spawns in winter, whereas the latter breeds in summer.

Similar to that in *P. scolops*, the growth of small oocytes of *Golfingia pugettensis* (Rice 1975) is arrested during the breeding season, and after the large eggs are spawned the small eggs appear to develop to maturity. In *P. scolops* large eggs (16×10^5 /ml CF) as well as sperm clusters (30×10^5 /ml CF) were observed in November and December (Figure 10). It is possible that a minor spawning had occurred 2 months after the major spawning. However, the possibility that the mature gametes may be either resorbed or phagocytized later instead of being spawned cannot be overlooked.

Hitherto no seasonal study of gametogenesis in any sipunculan based on the density estimation of gametes has been made, so observations in this study cannot be compared with those of other studies.

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